**Format for ANSWERING REVIEWERS**

September 23, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 18874-Review.doc).

**Title:** Role of Helicobacter pylori infection in pathogenesis of gastric carcinoma

**Author:** Rong-Guang Zhang, Guang-Cai Duan, Qing-Tang Fan, Shuai-Yin Chen

**Name of Journal:** *World Journal of Gastrointestinal Pathophysiology*

**ESPS Manuscript NO:** 18874

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Major comments.

1. As this manuscript does not have any figures and tables, it is difficult understand this review. The authors should use several figures and tables to assist the readers to understand well.

We have used 2 figures and 1 table in the revised manuscript.

2. AZ-521 cell line was used as a gastric adenocarcinoma cell line, but not a human gastric epithelial cell line. Recently, AZ-521 cell line has been found as same as a duodenal cancer cell line HuTu80, so authors must delete sentences about AZ-521.

We have deleted the sentences about AZ-521.

3. There are lots of overlapped description, e.g. the first part of the first paragraph of "Pro-carcinogenic responses". The authors should shorten this review.

We have deleted the overlapped description and shorten this review.

Minor comments.

1. Abbreviations must be used after full term first, such as VacA and OR. Please do not use 2 abbreviations for one term, such as TIVSS and T4SS.

We have used all the abbreviations after full term first, used only one abbreviation for one term.

2. There are lots of mistakes, so the authors must check those. e.g. They wrote "Studies have been conducted focusing on polymorphism of the main virulence factors, such as cagA, vacA, oipA, iceA, hopA, etc." in "H. pylori genetic variability". However, they wrote about hopQ in the following sentences. Is hopQ correct, but not hopA ?

We have checked the manuscript and corrected the mistakes, e.g. used the hopQ to take the place of hopA.

In addition, we have made more revisions as follows with reference to the file "Revision on the manuscript (No. 18874)":

[Line 7] added "Running title: Zhang RG *et al.* *Helicobacter pylori* and gastric carcinoma"

[Line 15-17] added "Author contributions: Zhang RG performed the review and wrote the paper, Duan GC conducted the review and inspected the manuscript, Fan QT and Chen SY took part in literature retrieval and analysis of the reported research findings."

[Line 19] added "Conflict-of-interest statement: No conflict-of-interest exists"

[Line 47-52] added "Core tip: It is important to gain further understanding of the pathogenesis of *H. pylori* infection for developing more effective treatments for this common but deadly malignancy. The recent findings on the bacterial virulence factors, effects of *H. pylori* on epithelial cells, genetic polymorphism of both the bacterium and its host, and the environmental factors for gastric cancer are discussed with focus on the role of *H. pylori* in gastric carcinogenesis in this review.
"

[Line 81-85] deleted "*H. pylori* has a number of virulence factors which influence colonization and disease severity. Because most *H. pylori* infections do not cause cancer, *H. pylori* heterogeneity has been investigated to identify possible virulence factors. " , added "*H. pylori* heterogeneity has been investigated to identify possible virulence factors owing to the fact that most *H. pylori* infections do not cause cancer."

[Line 89-90] added "a highly immunogenic protein," , deleted ", a 37-kb DNA segment," ,

[Line 123] deleted "VacA" , added "Vacuolating cytotoxin A (VacA)"

[Line 136-143] deleted "It was shown that human gastric epithelial cells (AZ-521) are highly susceptible to VacA-induced cell death. VacA-induced death of these cells is a caspase-independent process that results in cellular release of histone-binding protein high mobility group box 1 (HMGB1), a proinflammatory protein. These features are consistent with the occurrence of cell death through a programmed necrosis pathway, suggesting that VacA augments *H. pylori*-induced mucosal inflammation in the human stomach by causing programmed necrosis of gastric epithelial cells and subsequent release of proinflammatory proteins, and may thereby contribute to the pathogenesis of GC[28]. "

[Line 175-179] deleted "*H. pylori* gamma-glutamyl transpeptidase (GGT), as a virulence factor, play important roles in *H. pylori* colonization and cell death induced by *H. pylori* infection[42]. GGT appears in outer membrane vesicles[43], facilitate interactions with host cells. GGT increases IL-8 secretion and hydrogen peroxide production in epithelial cells and as a pathogenic factor, associated with H

pylori-induced peptic ulcer disease[44]. ”

[Line 179-182] added “*H. pylori* gamma-glutamyl transpeptidase (GGT) appears in outer membrane vesicles, facilitate interactions with host cells. GGT increases IL-8 secretion and hydrogen peroxide production in epithelial cells and associated with H pylori-induced peptic ulcer disease[41-43].”

[Line 195-196] added “The roles of the main virulence factors in pathogenesis of *H. pylori* infection are as shown in figure 1[6].”

[Line 197-207] added “Figure 1 The roles of the main virulence factors in pathogenesis of H. pylori infection. H. pylori colonization is facilitated by adherence to gastric epithelial cells mediated by BabA and SabA binding Leb and Lewis x/a respectively. The CagA protein is delivered into gastric epithelial cells through T4SS, and then tyrosine phosphorylated at EPIYA sites by Src and Abl kinases. CagA contributes to myriad signaling alterations, which profoundly influence host cell physiology with pathobiological effects including disruption of intercellular junctions, loss of cell polarity, the promotion of inflammation, dysregulation of proliferation and apoptosis. The VacA inducts mitochondrial damage, cytoplasmic vacuolation, apoptosis and immune suppression[6].”

[Line 210] added “The gastric epithelium consists of a monolayer of cells covered by mucus.”

[Line 224] deleted “As described above,”

[Line 254-255] deleted “*H. pylori* infection has proven difficult to cure despite intensive antibiotic treatment.”

[Line 302-305] deleted “*H. pylori* infection typically results in chronic superficial gastritis, followed by atrophic gastritis, intestinal metaplasia, dysplasia and adenocarcinoma[69]. The *H. pylori*-related chronic gastritis is linked to cancer development[70]. However, it has been reported that only 1%-3% of *H. pylori* infected persons develop GC[71]. ”

[Line 302-305] deleted “GC development is thought to be a multifactorial and stepwise process involving an array of etiological factors, genetic and epigenetic modifications and environmental factors.”

[Line 342] deleted “hopA, etc”, added “hopQ”.

[Line 379] deleted “Certain host factors also influence outcome of *H. pylori* infection.”.

[Line 447] added “as shown in table 1”.

[Line 452-457] added “Table 1 Association between cagA status and tobacco smoking

” and “ [a] Adjusted for sex, age (< = 50 years, >50 years), average of pure ethanol (g/day) (Never, moderate, high), consumption of proton pump inhibitors in the days prior to endoscopy (Yes/No), consumption of non-steroid anti-inflammatory drugs (Yes/No), and clinical presentation of upper digestive tract haemorrhage (Yes/No) [98].”

[Line 494-495] deleted “The objective of this review was to summarize our current understanding of the multiple aspects of the role of *H. pylori* infection in gastric carcinogenesis.”

[Line 502-503] added “as shown in figure 2.”

[Line 505-511] added “Figure 2 The pathogenesis of *H. pylori*-associated GC**.** The pathogenesis of *H. pylori*-associated GC is a multi-factorial process, its development depends on a combination of host, bacterial and environmental factors, and the pathological changes might progress in steps.”

[Line 513-514] added “ACKNOWLEDGEMENT Research support from the China Postdoctoral Science Foundation (No. 200801273)”

[Line 605-607] deleted “28. Radin JN, González-Rivera C, Ivie SE, McClain MS, Cover TL. Helicobacter pylori VacA induces programmed necrosis in gastric epithelial cells. *Infect Immun* 2011;**79**:2535-2543 [PMID: 21482684 DOI: 10.1128/IAI.01370-10]”

[Line 745-761] deleted the reference 69-71

All the revision described above have been made as shown in the file "18874-Revision made on the manuscript", resulting in the file named "18874-Revised manuscript".

Best wishes,

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